AN EVALUATION OF THE TRIBAL POSITION OF *CHAMAELIRIUM LUTEUM* (L.) GRAY BASED ON CHROMOSOMAL NUMBER

BY

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AN EVALUATION OF THE TRIBAL POSITION OF *CHAMAELIRIUM LUTEUM* (L.) GRAY BASED ON CHROMOSOMAL NUMBER

A Research Paper

Presented to

the Graduate Council of

Austin Peay State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

in Biology

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by

Judith Helene Slazyk Johnson

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To the Graduate Council:

I am submitting herewith a research paper written by Judith Helene Slazyk Johnson entitled "An Evaluation of the Tribal Position of Chamaelirium luteum (L.) Gray based on Chromosomal Number." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science in Biology.

Edward W. Chester
Major Professor

Accepted for the Council

Wayne E. Stamps
Dean of the Graduate School

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CHAPTER I

INTRODUCTION

Chamaelirium is a monotypic genus of the family Liliaceae. It is known by several common names including Devil's bit, Fairy-wand, Rattlesnake-root, and Blazing-star. Fernald (1950) notes that the name was taken from the Greek, Chamai, on the ground, and leirion, lily. However, this name was apparently taken from a dwarf plant and is not indicative of the species.

Chamaelirium luteum is a dioecious monocot. It is recognized by a graceful raceme-like inflorescence arising from the axillary buds of the thick rhizome in both staminate and pistillate plants. The flowers are without bracts and appear acropetally. Staminate and pistillate perianths are similar in size and shape, with three white sepals and three white petals. The male plant has three short and three long stamens. The flowers turn yellow upon drying. The pistillate inflorescence is characteristically shorter, thicker, and elongates at a slower rate than that of the staminate. The ovulary walls develop chlorophyll quite early, giving the female flowers a greenish appearance (Silliman, 1957).

The stem leaves of Chamaelirium differ greatly from the basal leaves. The basal leaves vary from two to twenty-one per plant with an average number of between seven and eleven. They are arrenged in a rosette, are oblong, spatulate, and with obtuse apicies. Each basal leaf has a prominent midrib with four to six lesser veins running parallel to the margin of the leaf. The smaller veins tend to

form a network from the midrib, an arrangement more often found in dicotyledons. The stem leaves become smaller as they progress up the stem and are bract-like and sessile near the inflorescence. Stem leaves range in number from nine to twenty-eight in the staminate plant, with proportionately more occuring on the longer pistillate stalk. The stalk of the staminate plant is 2 - 7.5 decimeters in height while the length of the pistillate stalk is 2 - 12 decimeters (Silliman, 1957).

The tuberous rhizome is usually three to four centimeters in length with a "bitten off" look, hence the name Devil's bit. White, fleshy, fibrous roots arise from the upper area of the short rootstock (Silliman, 1957).

Although described as a dioecious plant, polygamodioecious specimens have been found. Staminate plants are usually more numerous than pistillate (Silliman, 1957). Chamaelirium flowers from May through July.

The habitats in which this plant is found include rich woods and meadows in a variety of soil and elevational conditions. The distribution extends throughout Eastern United States from Massachusetts to Florida. It is found as far north as Southern Ontario and Michigan, and as far west as Illinois.

Most taxonomists agree that Chamaelirium is a member of the family Liliaceae, however, there is disagreement as to its position among the various tribes. Watson (1879) divides the Liliaceae into 16 tribes, placing Chamaelirium in Tribe XIV, Helonieae.

Bentham and Hooker (1883) place Chamaelirium in Tribe XVII, Narthecieae. They describe 20 tribes of Liliaceae. Engler and Prantl (1889) place Chamaelirium in the sub-family Melanthoideae, Tribe II, Helonieae. They prefer 11 sub-families and 31 tribes. Krause (1930) places Chamaelirium in the tribe Helonieae. Hutchinson (1934) divides the Liliaceae into 28 tribes and 3 other families. He places Chamaelirium in Tribe I, Heloniadeae of the Order Liliales. Table I lists the historical tribal classifications of Chamaelirium luteum.

Claus (1944) in a study of the taxonomy of the Liliaceae based on phytoconstituents compared the taxonomic systems of Bentham and Hooker, Engler and Prantl and Hutchinson, finding the system of Hutchinson to be most satisfactory. However, he recommended removal of Chamaelirium from its present classification based on the presence of glycosides. Other members of the sub-family Melanthoideae (Engler and Prantl, 1889) which he examined include the genera Tofieldia, Veratrum, Zygadenus, Schoenocaulon, Amianthium, Gloriosa, Androcymbium, Colchicum and Bulbocodium which were found to contain alkaloids but no glycosides.

Sato (1942) did an extensive karyotypic analysis of many of the Liliaceae and found that the diploid number was 24 in all members of the tribe Heloniadeae examined. Chamaelirium was not included in this study. Of those members of the tribe Narthecieae analysed, the diploid number was found to be 30.

Previous work on Chamaelirium luteum includes a biological study

TABLE I

HISTORICAL TRIBAL CLASSIFICATION OF

CHAMAELIRIUM LUTEUM

AUTHOR	DATE	TRIBE
Watson	1879	Helonieae
Bentham and Hooker	1883	Narthecieae
Engler and Prantl	1889	Helonieae
Krause	1930	Helonieae
Hutchinson	1934	Heloniadeae

(Silliman, 1957) which extensively treats the aspects of morphology, histology and embryology.

A review of the literature indicated the absence of published data of chromosomal numbers in Chamaelirium. The purpose of this study was to determine the chromosome number of Chamaelirium luteum in order to further evaluate its tribal position.

CHAPTER II

METHODS AND MATERIALS

The plant material used in this study was collected in April, 1969 from Todd County, Kentucky, two miles south of Allegre, and maintained in the Austin Peay State University greenhouse. The original habitat was a moist, wooded ravine.

The technique used was a modification of the oxyquinoline-aceto-orcein squash method developed by Tjio and Levan (1950) and used by Kawano and Iltis (1963) in a study of the genus Smilacina.

Fresh root-tips were placed in 0.002 M 8-hydroxyquinoline solution at 17 to 20 degrees centigrade for 3 hours. This pre-treatment is necessary to clear the cytoplasm, allow separation of the middle lamella causing the tissue to soften, and to allow spreading of the chromosomes during squashing. Cooling seems to accelerate the pre-treatment (Sharma and Sharma, 1965).

The root-tips were then fixed in Farmer's ethanol acetic acid (3:1) solution for 15 minutes. Fixation is necessary to kill the cells without distortion of the structures which are to be studied. Fixation is optimum when visibility of structures is increased and morphological details are clarified. The characteristics most desired in a fixative are qualities which immediately kill the tissue, precipitate the chromatin, stop autolysis of proteins, prevent decomposition and enhance the basophilia of the chromosomes (Sharma and Sharma, 1965).

The root-tips were stained for 24 hours in 2 per cent aceto-

orcein and then transferred to 2 per cent aceto-orcein-1 N HCl (1:1) solution for 5 to 10 minutes.

Orcein, the stain used in this study, was first used by La Cour in 1941 as a chromosome stain. It has a molecular weight of 500.488 and the chemical formula is $C_{28}H_{24}N_2O_7$, however its exact chemical structure is unknown. It is a deep purple color from the action of hydrogen peroxide and ammonia on the colorless orcinol. Most commonly, it is used as aceto-orcein in chromosome studies. In nature it is obtained from two species of lichens, Rocella tinctoria and Lecanora parella. It is also manufactured synthetically. It is felt that natural orcein is a more effective dye than the synthetic product (Sharma and Sharma, 1965).

Proper softening of the tissue is vital to the squashing technique. This is carried out in the 2 per cent aceto-orcein-1 N HCl solution after staining. This acid-stain solution dissolves the pectic salts of the middle lamella so that individual cells can separate and stains the chromosomes more deeply (Sharma and Sharma, 1965).

After softening, the root-tips were placed on a glass slide, mounted in a few drops of acetic acid (45 per cent)-glycerine mixture (9:1) for 20 seconds and gently heated over an alcohol lamp, taking care that the preparation did not boil. A cover slip was placed over the root-tips and they were squashed by applying pressure.

The technique of squashing is not only rapid, but also advantageous because single cells enlarge in volume providing a greater

area within which the chromosomes scatter and separate (Sharma and Sharma, 1964).

The squash preparations were scanned under oil immersion to find the area of mitotic activity. Sketches were made, using a camera lucida, for an accurate count of the chromosomes since they were found to be extremely tiny and difficulty was encountered in preparing thin squashes.

Photomicrographs were made on 35 mm Eastman Tri-X film through a Unitron BU-13 microscope with a 40x objective and a 20x ocular. Prints were made at 800x.

CHAPTER III

RESULTS

Many squashes were made of the root-tips of several plants. The cells studied revealed that the diploid number of chromosomes in Chamaelirium luteum is 24. The camera lucida made an exact count easier and helped to insure accuracy. Photomicrographs of cells of Chamaelirium luteum containing the diploid number of 24 chromosomes may be seen in Figure 1.

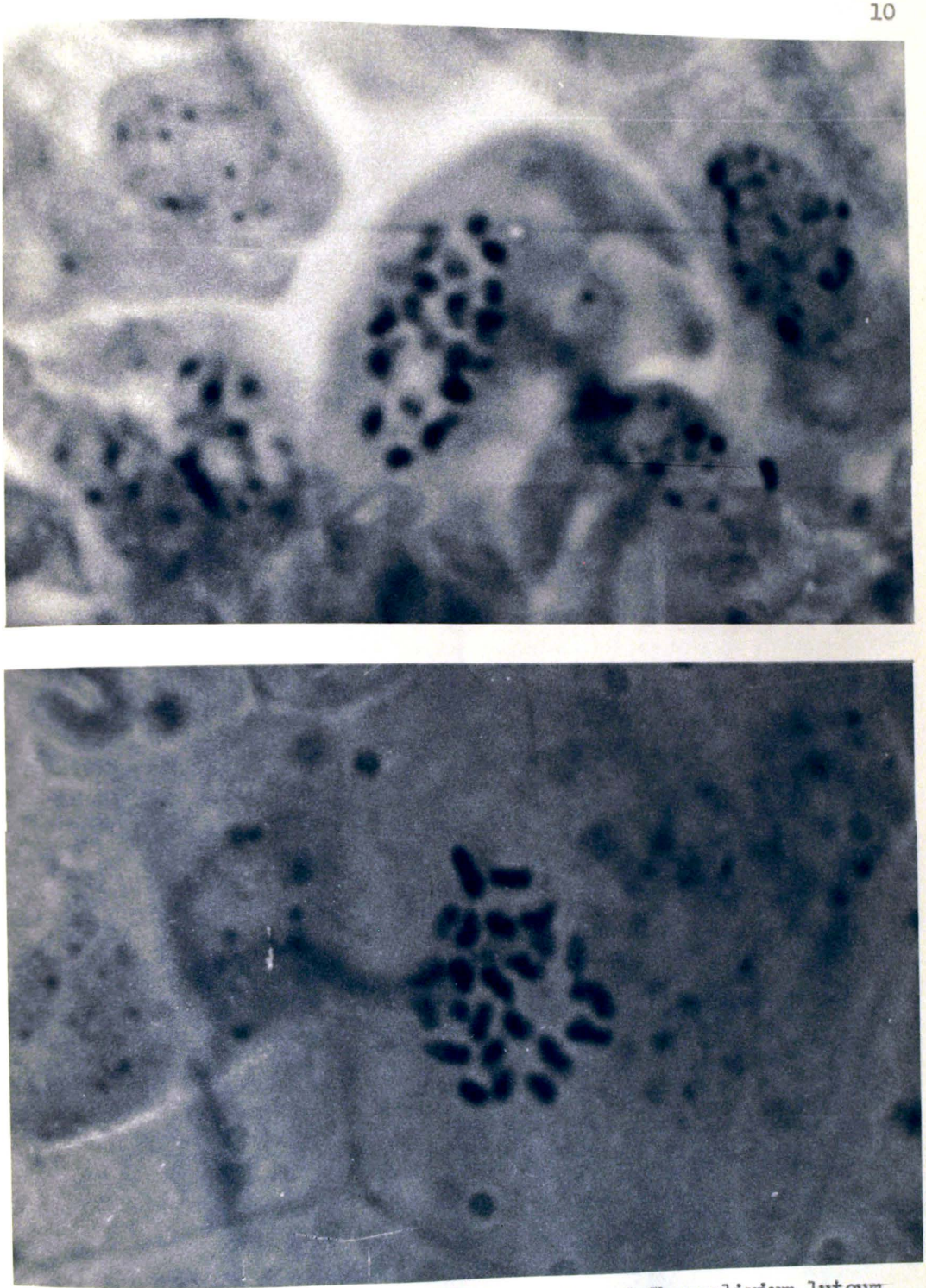

Figure 1: Photomicrographs of root-tip cells of Chamaelirium luteum containing the diploid number of 24 chromosomes.

CHAPTER IV

DISCUSSION

Results of this study seem to indicate that the proper tribal placement for Chamaelirium is the Heloniadeae according to the classification of Hutchinson or the Helonieae of Watson, Engler and Prantl, and Krause.

A study of the phytoconstituents of several members of the Liliaceae led Claus (1944) to the conclusion that taxonomy based on phytoconstituents should parallel and support taxonomy based on morphological characteristics. The chemical constituents found in the Liliaceae include glycosides (heart stimulants) and alkaloids (heart depressants). He found that twelve genera of Liliaceae contain glycosides, some of which are saponins. Chamaelirium is one of these. Sixteen of the genera analysed show the presence of alkaloids. Chamaelirium, however, is classified by Engler and Prantl, Bentham and Hooker, and Hutchinson with these plants of an alkaloidal nature. Since in most cases taxonomy based on phytoconstituents seems to substantiate taxonomy based on morphological characteristics and since there is no chemical relationship between Chamaelirium and the other genera of its present classification, Claus would recommend its reclassification.

Sato (1942) analysed the karyotypes of seventy-two genera of Liliaceae and other allied families. The diploid number of chromosomes of Chionographis japonica and Japonolirion osense, members of the tribe Heloniadeae, was 2n = 24. The diploid number of chromosomes

found in Tofieldia nutans and T. nuda was 2n = 30 and in the tetraploid Tofieldia japonica the chromosomes numbered 60. These genera are members of the tribe Narthecieae. Sato concluded that the systems of classification proposed by Hutchinson, Watson, Engler and Prantl, and Krause are more acceptable than the classification proposed by Bentham and Hooker.

Sato stated that the classification system of Hutchinson is more suited to the results of the karyotypic analysis of the Liliaceae than that of Engler and Prantl. Hutchinson stresses the type of inflorescence in his classification while Engler and Prantl's system is based on the superior and inferior position of the ovary. Hutchinson's system seems to be a less artificial means of classification and for this reason is prefered by Sato.

In the light of the study by Sato, my results correlate with the placement of Chamaelirium in the tribe Heloniadeae as proposed by Hutchinson, or the Helonieae of Watson, Engler and Prantl, and Krause.

The fact that members of the tribe Narthecieae, under Bentham and Hooker's classification, have a diploid number of 30 chromosomes would indicate that this is an incorrect classification for Chamaelirium.

These results seem to indicate that classification by phytoconstituents alone may not be entirely reliable, although in many cases phytoconstituents may reinforce morphological and vegetative data. A karyotypic analysis is a definite aid in studying the phylogeny of plant groups. However, further work on other populations of

Chamaelirium from broader geographic ranges would be highly desirable for a more complete study.

CHAPTER V

SUMMARY

A karyotypic analysis of Chamaelirium luteum using a modification of the oxyquinoline-aceto-orcein squash method developed by Levan (1950) was carried out. A diploid chromosome number of 24 was determined with the aid of a camera lucida and photomicrographs. This data, in the light of studies by Claus (1944) and Sato (1942) led to the conclusion that Chamaelirium luteum should remain in the tribe Heloniadeae as formerly classified by Hutchinson.

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